

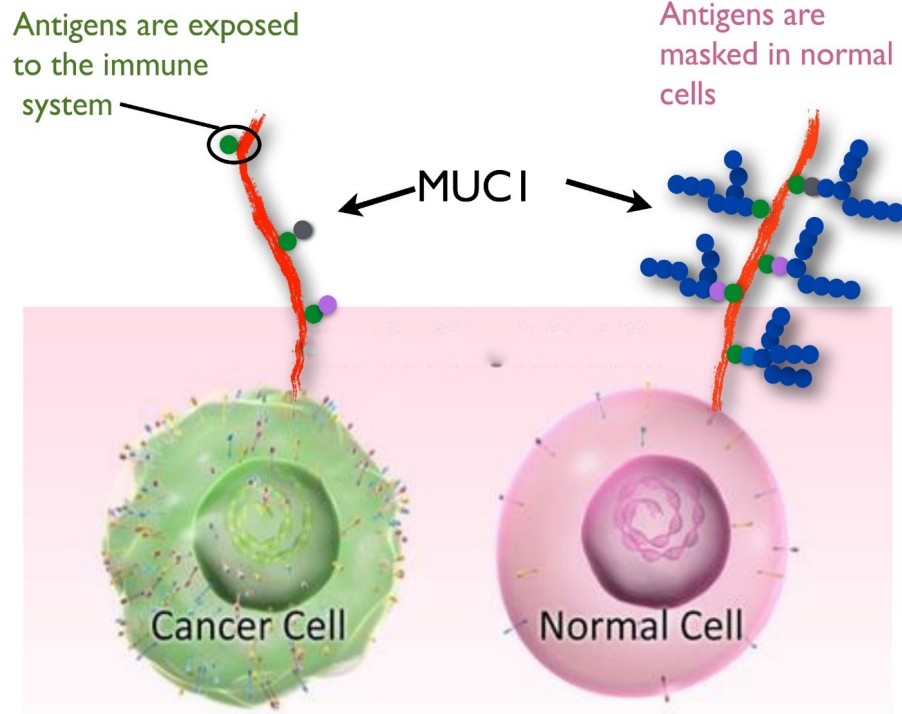
2nd Regular Meeting of the DIRNANO consortium; ESR 14 progress report

H2020-MSCA-ITN-DIRNANO project no. 956544, entitled: “Directing the immune response through designed nanomaterials”.

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Associate Professor
Universidad de La Rioja*

Mucin-1; an excellent cancer biomarker... but also an excellent antigen?



Nat. Rev. Cancer **2015**, *15*, 540–555

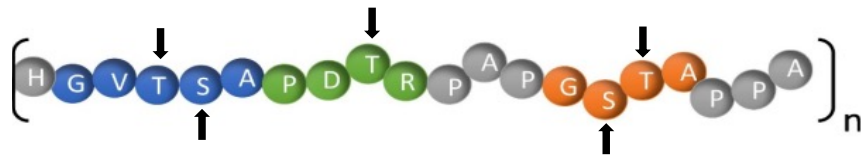
- *MUC-1* is a glycoprotein with a key role in cancer development
- *Different post-translational modifications in healthy and cancer cells*
- *Diagnostic biomarker in several cancers*
- *Promising vaccine candidate*

However...

- *Low immunogenicity*
- *Low stability*

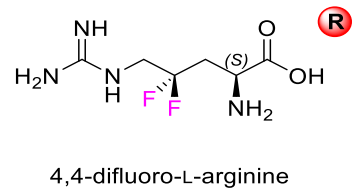
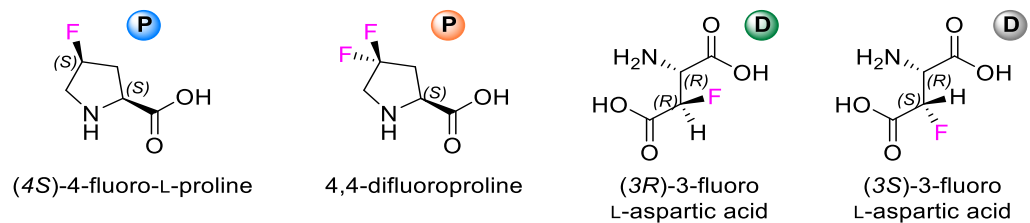
Developing Mucin-1 to an ideal cancer antigen using unnatural amino acids

MUC-1 tandem repeat domain

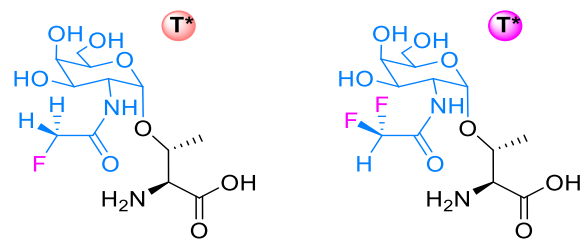


Unnatural fluorinated derivatives

Fluorinated amino acids:

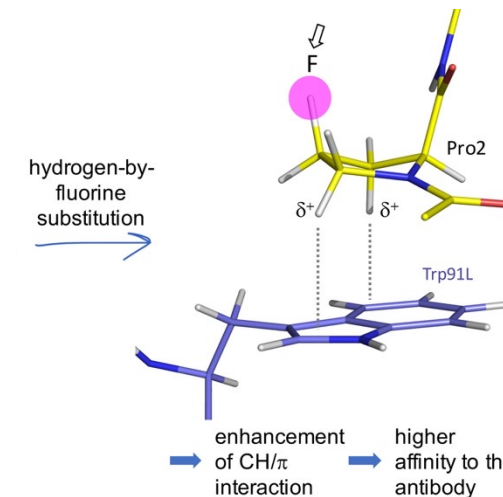


Fluorinated glycosylamino acids:



- Increased stability
- Increased immunogenicity
- Enhanced binding affinity

Fluorine substitution to increase the binding affinity of the antibody

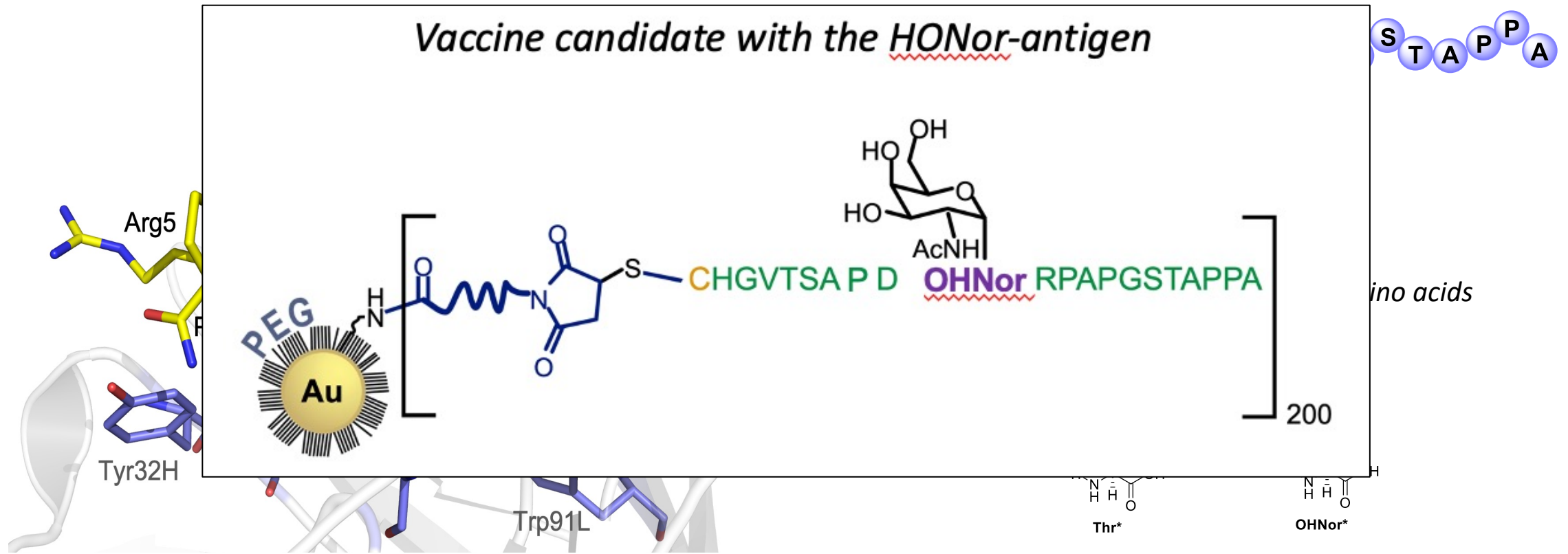


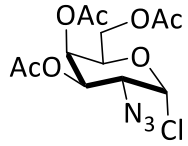
Corzana et al. *J. Am. Chem. Soc.* **2017**, *139*, 18255

Structure of the Hydroxy-Norvaline & Utilization in a nano-vaccine candidate; PEG-coated AuNPs

Crystal structure of the complex Ab-HONor-antigen

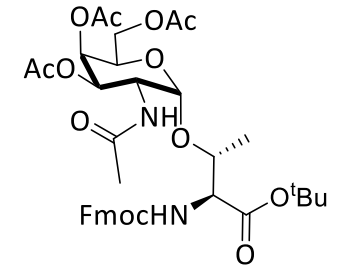
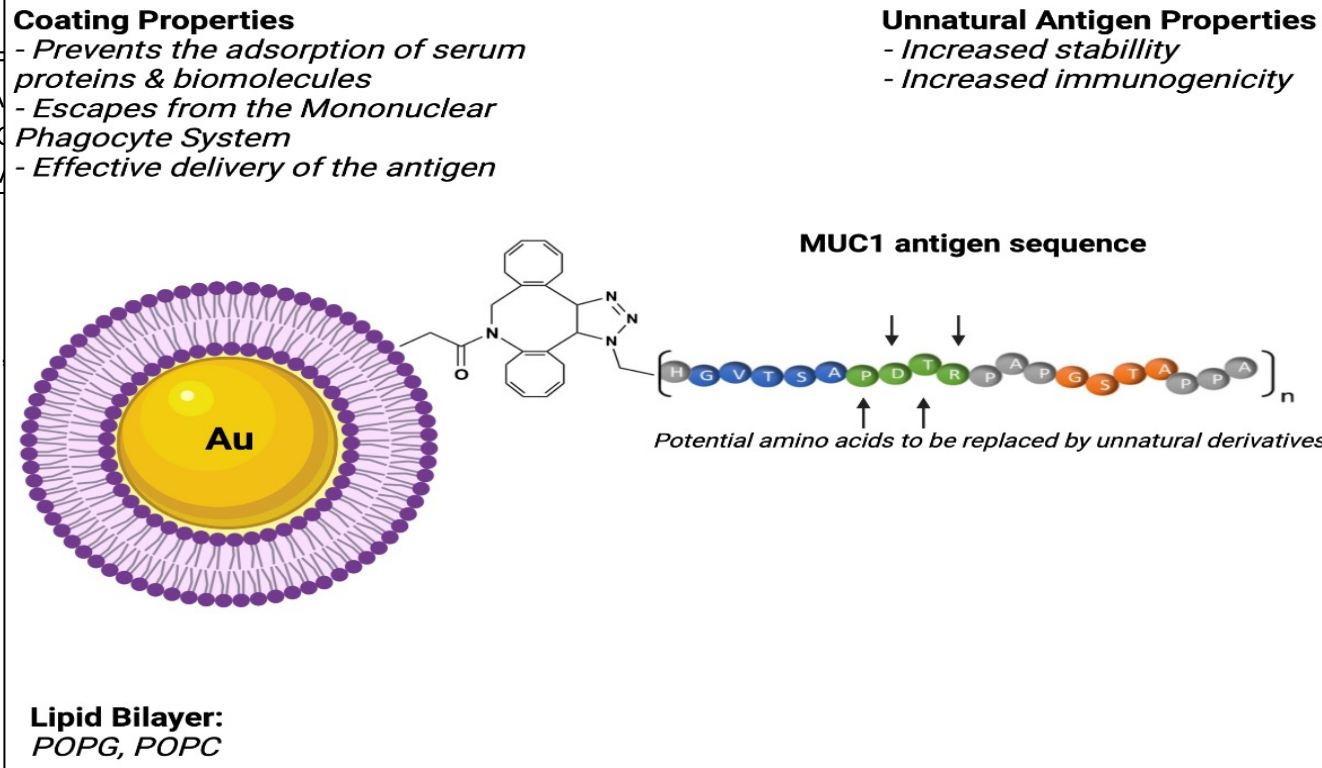
MUC-1 sequence



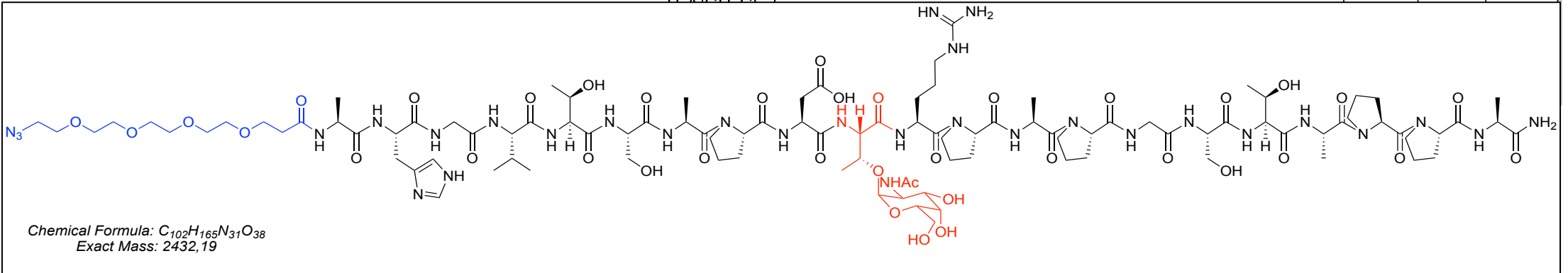
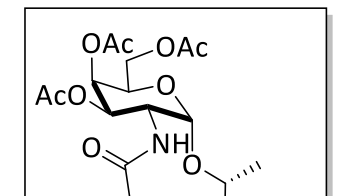


Org. Lett. 2011, 13

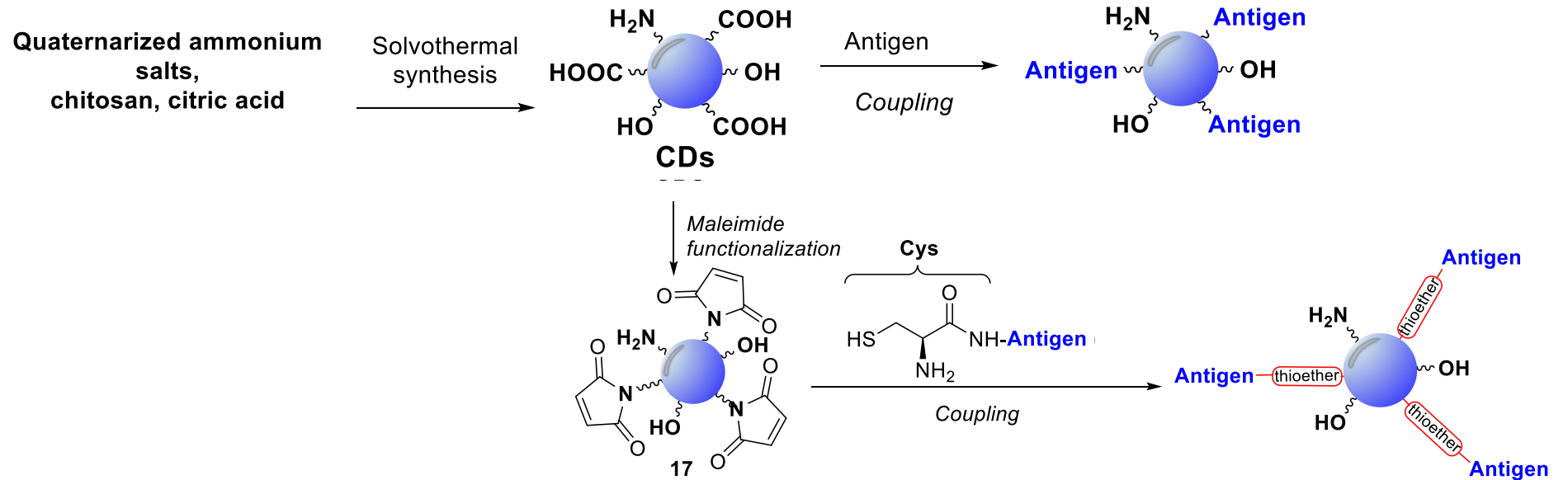
Future unnatural derivative & Utilization in a nano-vaccine candidate; POPG/POPC-coated AuNPs



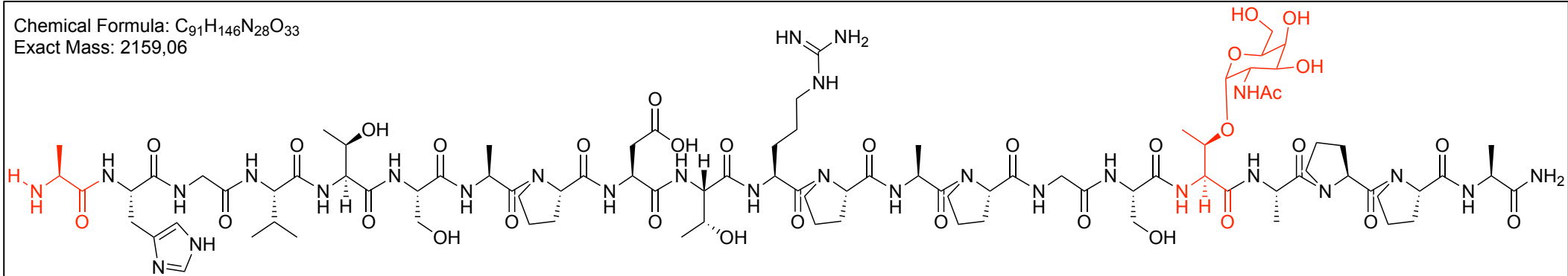
TFA/CH₂Cl₂
25 °C, 3h
95%



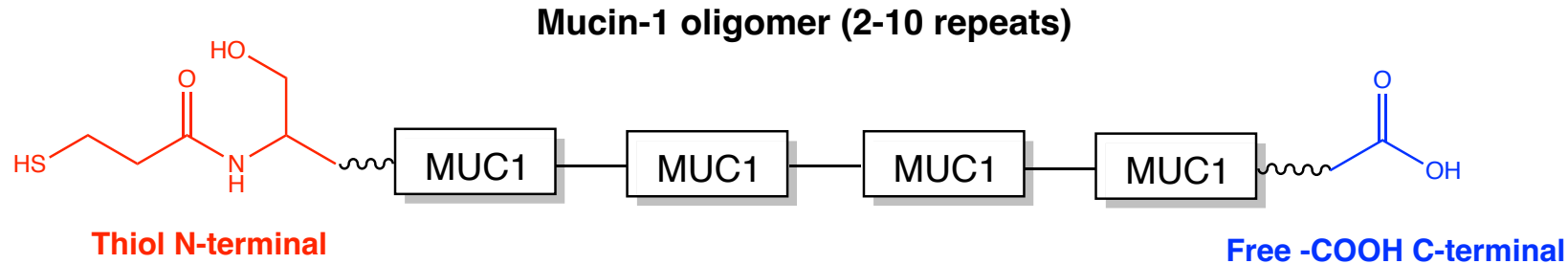
Utilization of Carbon Dots for the development of an another nano-vaccine candidate



Chemical Formula: $C_{91}H_{146}N_{28}O_{33}$
Exact Mass: 2159,06



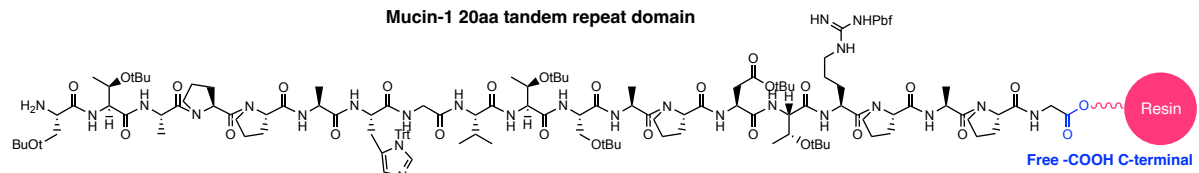
Mucin-1 Oligomer Formation



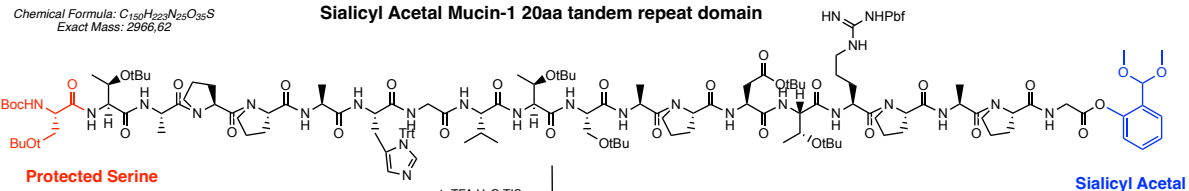
- *Development of MUC1 oligomers (2-10 repeats) of the 20aa tandem repeat domain*
- *Idea based on the MUC1 100mer peptide vaccine¹*
- *Protocol based on, and adapted from a previous research work²*
- *Specific conjugation through the functionalised Thiol N-terminal*
- *Enzymatic post-synthetic glycosylation*

1. Kimura *et al. Cancer Prev. Res.* **2013**, 6(1), 18-26
 2. Xu *et al. Chem. Commun.*, **2013**, 49, 6200

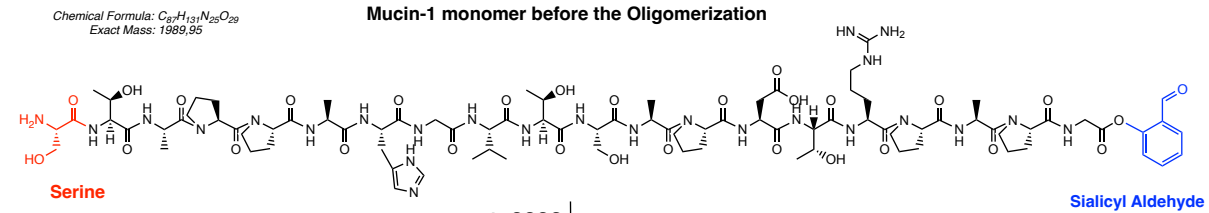
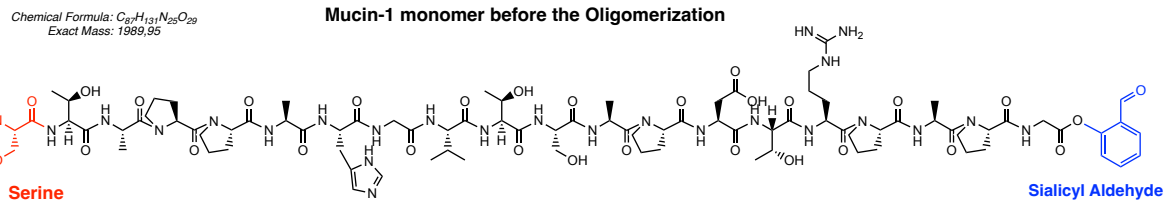
Synopsis of the experimental protocol of the Mucin-1 oligomerization



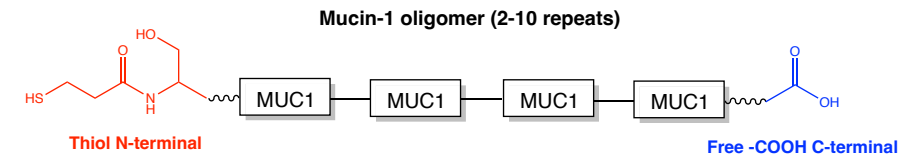
1. Boc DIEA (in NMP) DMF, RT, 30mins
2. HFIP:CH₂Cl₂ (2:8, v/v) RT, 1hr
3. Sialicyl Acetal PyBoP/HBTU CH₂Cl₂, RT, 1hr



1. TFA:H₂O:TIS (95:2.5:2.5, v/v) RT, 2-3hr
2. ????
3. HPLC purification under acidic conditions



1. ????
2. Quenching
3. TFA:H₂O (95:5, v/v) RT, 10mins
4. Purification (Ultrafiltration/ Gel Filtration)



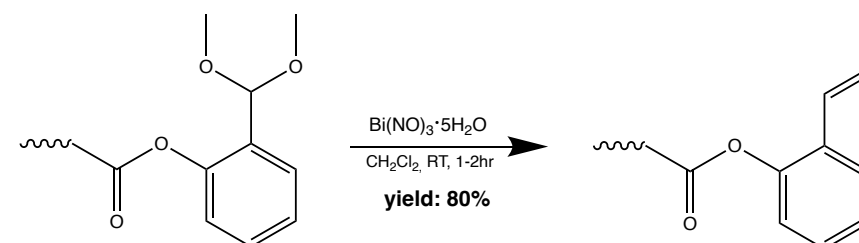
Xu et al. *Chem. Commun.*, **2013**, 49, 6200

Optimization of the acetal deprotection reaction

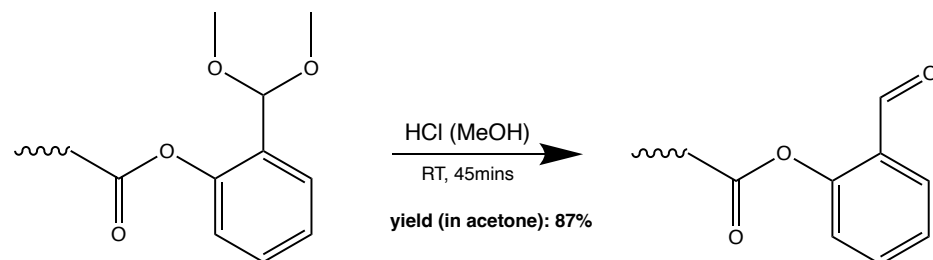
Catalysis by molecular Iodine in acetone¹



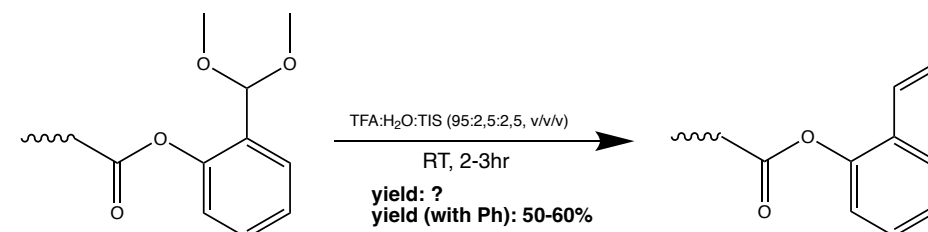
Acidic deprotection by $Bi(NO_3)_3 \cdot 5H_2O$



Acidic deprotection by HCl in acetone³

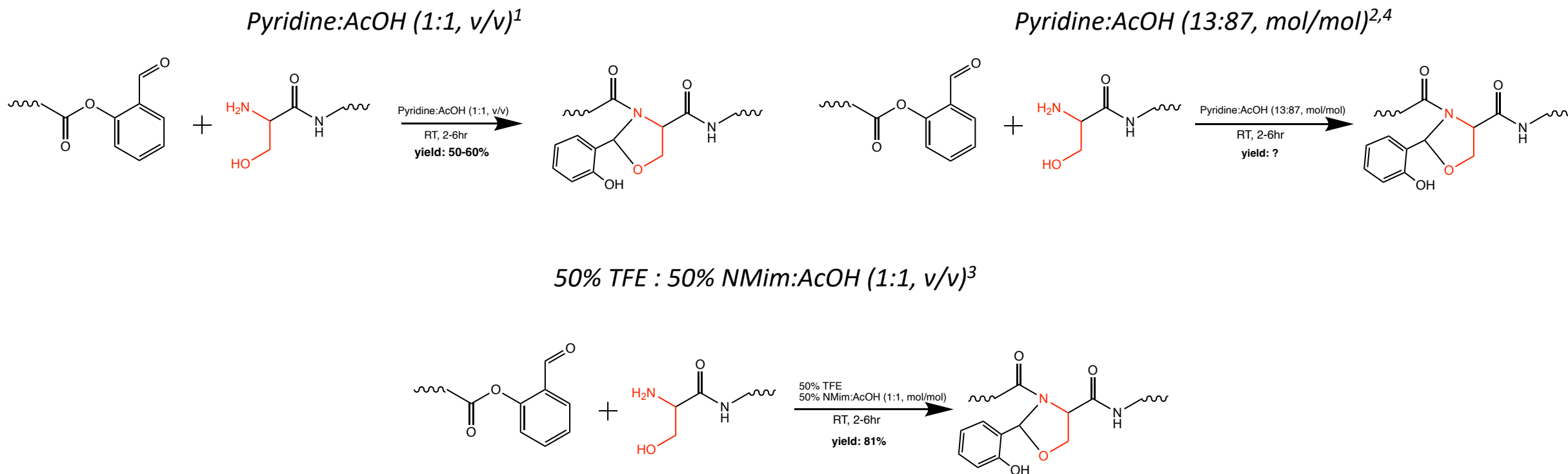


Scavenger replacement (TIS) may improve the yield



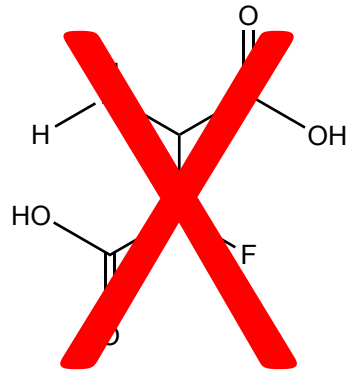
1. Sun et al. *J. Org. Chem.* **2004**, 69, 25, 8932–8934
2. Eash et al. *J. Org. Chem.* **2000**, 65, 8399-8401
3. Baar et al. *J. Chem. Educ.* **2005**, 82, 7, 1057

Optimization of the ligation reaction

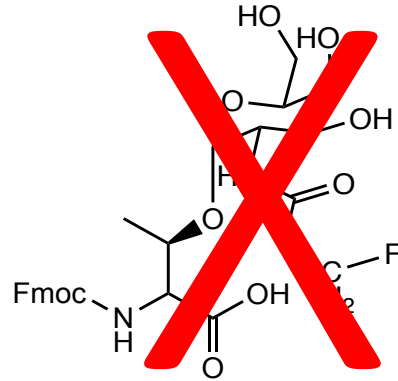


1. Xu et al. *Chem. Commun.*, **2013**, 49, 6200
2. Venkatesan and Suryanarayana *Nature*, **1956**, 178, 1345–1346
3. Tam and Miao *J. Am. Chem. Soc.* **1999**, 121, 39, 9013–9022
4. Xu et al. *Tetrahedron*, **2020**, 76(18), 131143

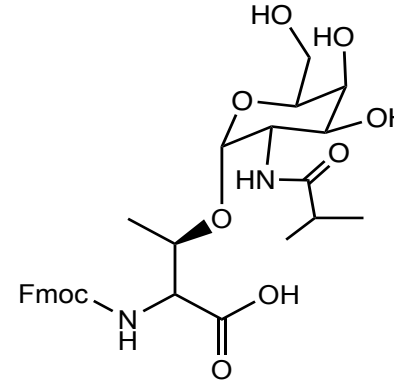
Other unnatural & fluorinated compounds to be prepared or under investigation



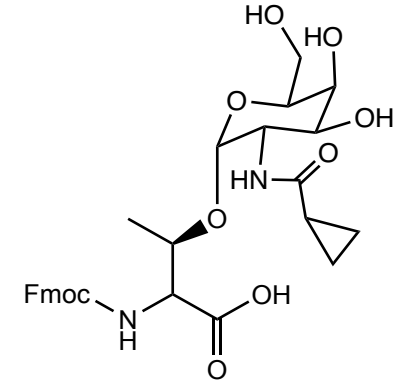
Fluorinated Aspartic Acid



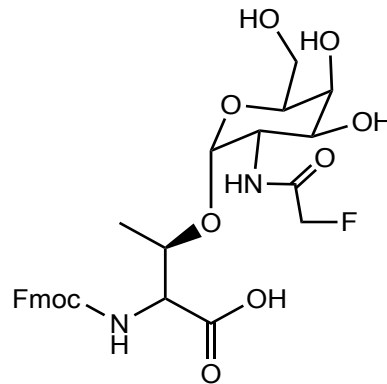
Fluorinated Propyl
Tn-Threonine



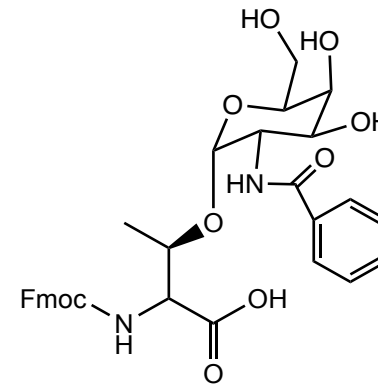
Iso-propyl Tn-Threonine



Pyramidic Tn-Threonine



Fluorinated Acetyl Tn-
Threonine



Benzyl Tn-Threonine

Acknowledgements



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Directing the immune response through
designed nanomaterials

H2020-MSCA-ITN-2020



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Directing the immune response through
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*Thank you a lot,
for your attention!*